

Cavalli ↓

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Dear Josh,

This is a response to your request of writing the history of how I became interested in microbial genetics. The beginnings go back to 1940-41. I was a medical student and I spent the summer holidays between the second and third year of Medical school (total six years) working with Giovanni Magni in the microbiology-hygiene laboratory of the province of Como, north of Milan. At the beginning of the third academic year the professor of pathology, Emilio Veratti, gave us a book, "The Genetics of Microorganisms", a symposium made in America shortly before the war. The book belonged to Adriano Buzzati-Traverso; his name was written in his typical hand-writing on the first page, but I did not know him then, and Veratti told us he had received the book from Adriano, who had suggested to him to give it to students who might be interested. Veratti was an old man, sincerely interested in research, who gave us also on other occasions good advice. I am saying "we" because Giovanni Magni and I were working closely together at the time.

Giovanni and I shared the translation into Italian of the book on microbial genetics, which was fairly naive but had some interesting ideas. I remember only a paper by Mary Bunting, on prodigious color mutations; anyhow, we later published in an Italian scientific journal a summary of the book and of whatever other literature we were able to find.

Adriano was in the army, in Lybia, but was dismissed because of sickness- probably an allergy. He returned to his university job in Pavia at the beginning of 1942 and started giving a course in Genetics. When I heard about it, I decided to follow his Genetics course for Natural Science students, and in fact prepared a detailed syllabus, on his request, based on his lectures as well as on the books on which they were based. They were excellent books like Sturtevant and Beadle, Waddington, and others. Through Adriano I also managed to have the first books on statistics, Snedecor, and Tippett, which I had longed for, but never had access to. There were no books on statistics in Italian of any value at the time. A book on which I had studied Mendel's laws in the first year of medical school had turned me off completely. During the war there was of course no way to obtain English or American books and journals.

Contact with Adriano was very exciting, and I started thinking I might orient my choice towards genetics. In the microbiological lab where Giovanni and I worked in the summers we started a genetic problem: mutations induced by ultraviolet in *Bacillus anthracis*. There was already some evidence at the time, if I remember correctly, that ultraviolet might be mutagenic. There were of course no germicidal lamps, but plain mercury lamps. The choice of the organism was probably suggested by the director of the Como laboratory, G. Banezzi, whose main contribution was the idea that one cannot believe that a variant strain is a mutant

unless there has previously been a single cell isolation under microscopic control (by the micromanipulator). He taught us the technique, and this was fine; the choice of *B. anthracis* was silly, because it forced us to great precautions. At the time there were no antibiotics to rely upon if something went wrong. We treated heavily with UV some isolates, and tested their fermentations, and virulence. We found changes in both, which greatly surprises me in retrospect, because we tested very few colonies; however, we did publish the results, fortunately in Italian only. It is nearly impossible to count on plates *B. anthracis*, which ordinarily forms very long chains, so there was no simple way of estimating survival frequencies.

A consequence of these investigations was an increased interest in the measurement of virulence, mainly in response to the standard attitude of bacteriologists of the time, who claimed virulence cannot be measured. We noted a regular increase in time of death of mice injected with increasing dilutions of *B. anthracis*, and thought of an explanation in terms of logarithmic growth and the need to reach a lethal concentration of germs, independent of the injected dose.

In order to be able to discuss this explanation with a biologist who was not completely put off by a mathematical formula, Giovanni and I went in the summer '42 to Frankfurt, with a short term scholarship, to work with Richard Prigge. The suggestion had come from Veratti, and proved excellent. Prigge was very nice to us and let us use hundreds of mice (all of the same weight and sex, plus minus half a gram) and gave us help to carry our experiments on virulence, this time on pneumococci. Results were dutifully published on *Zentralblatt für Bakteriologie*. Originally the intention was to do genetics of virulence, but we never came to it, because after we left Prigge's laboratory the chance of working so well never occurred again.

Adriano suggested that we should, during our stay in Germany, join him in Berlin, where he was working with Timofeeff-Ressovsky on radiation and also on population genetics of *Drosophila*, and so we did. That stay was completely determining for my research career. The brilliant personality of Timofeeff had a deep impact on me. He convinced me completely that genetics was the best choice I could make, and on my return to Pavia I became a regular "intern" in Adriano's lab. I did some research work of no great interest in cytogenetics, radiogenetics, and finally (with greater satisfaction) population genetics of *Drosophila*. I chose to do a thesis on the killing of bacteria by X-rays, however, in the hope of understanding something of the bacterial nucleus of *E. coli*. For this aim, I went to Rome State Institute of Health for a few months, where I found good support in terms of supplies, electron microscopy, and X ray. An M.D. thesis was supposed to be a minor effort, and I certainly made no discoveries. Most of my lab time between '43 and '45 was spent in *Drosophila* work with Adriano at a temporary location (Pallanza, on Lake Maggiore) chosen by him to avoid destruction of the lab by bombs. Actually, neither this place, nor, to say the truth, Pavia were touched by bombs. In this period I also tried my potential interest for a medical career by serving as M.D. in the local hospital. I was completely turned off.

In the North of Italy the war ended on April 24, 1945, with the arrival of allied troops. The Italian universities were in complete disarray, and there was no opening in sight. The first came in 1949, an assistant "professor" job with Adriano; at the time I was in England and not interested, but Giovanni took it. Thus at the end of

the war I gave up my M.D. position and started looking for some research position outside the University. I also wanted to marry Alba, whom I had met in Sept. '43 during field work in *Drosophila* population genetics at Adriano's country house.

In August 1945 I found a position at a pharmaceutical firm, a non-profit making organization, where I was supposed to work in the research department with a bright immunologist, E. Carlinfanti. The general director of the place (the Istituto Sieroterapico Milanese) was also the professor of microbiology at the University of Milan. He was an intelligent but unbalanced person and soon lost his job as director. I was asked, contrary to initial promises, to spend mornings bleeding blood donors; I developed skills I used later in field work, but certainly I did not enjoy this use of my time. In the afternoons I did some experimental and statistical work on immunology, but soon was able to have enough independence to come back to bacteria. Niccolo' Visconti came as a guest to the lab, and together we tested cross-resistance of *E. coli* B and B/r to X-rays and nitrogen mustard. I was also interested in understanding the physiology of the sensitivity to radiation of the B strain. At a time when radiations seemed to offer the only approach to the study of the bacterial nucleus, the behaviour of B sounded outrageous.

I had been very active trying to get a fellowship to go abroad; I managed to get one from the Italian National Research Council, and obtained it while I was in the army for conscription duty. I managed to get out of the army in April 1948. The fellowship was meant to last a year, but it could barely pay for three or four months of cheap accommodation in England. Fortunately I got a paid leave from my place in Milan, and the family could survive. We had one child, Matteo, at the time; Alba and Matteo staid in Milan. I still had a strong attraction for statistics, and for the genetics of quantitative characters; it seemed to me they were very important for evolution. K. Mather, then at the John Innes Horticultural Institution, at Merton near London was working on it; he was also a strong statistician, so I asked to spend my fellowship there. I worked in *Drosophila* quantitative inheritance and learned multivariate analysis in Mather's lab, between April and August 1948.

As you can see, I had been on and off bacterial genetics for six years, mostly off, and you may find my story a little irrelevant so far. But now comes the turning point. As you know, there was no bacterial genetics until 1946, and I was really waiting for it to be founded. I believe my first chance to read your CSH paper must have been when I was at Merton with Mather, because it took much time after the war for English and American journals to start coming again. To my best recollection, I must have read your 1947 article at Merton, when I was with Mather. I remember I had some difficulties understanding how the selection of BM and TL markers affected the *Lac* V1 segregation.

In August 1948 there was an international Congress of Genetics at Stockholm, and I decided to go and give a paper on the cross resistance work to nitrogen mustard and X rays with Visconti. Also Vernon Bryson gave a similar paper, independently. An almost incredible thing happened at Stockholm. I introduced myself to R.A. Fisher. I remember distinctly, on the steps of the lecture hall of the congress, and after I said the first ten words he offered me a job at Cambridge to work on bacterial genetics. My immediate reaction was divided. Naturally, I was extremely

pleased - I had chosen to go to Mather because I thought Fisher was too difficult for me to follow. Already at that time the great trio of founders of evolutionary theory: Fisher, Haldane and Wright had been deified. Perhaps, in fact, I had never considered asking to work in Fisher's or Haldane's lab, and had chosen Mather instead, also because Fisher and Haldane seemed like unapproachable gods. Thus it was simply incredible to be offered a job in Fisher's place. But I also had a concern; how could he offer me a job after I said no more than ten words to him? How could he take me seriously? I never asked him then or later, but I suspect he may have heard of me from Mather, with whom I had by then spent several months. Anyhow, I accepted on the spot.

On my arrival to Cambridge, on the first October of 1948, I had to order everything to start a bacterial genetics lab. I was able to begin doing something in Feb. '49. I got one fourth of the room ordinarily used for tea (as you know, an important ceremony in England) to make my lab, and another fourth for the kitchen. The University gave me a job less good than originally promised, which should have been that of Assistant Director of Research. In part, they enjoyed playing tricks to Fisher; in addition, they certainly were unhappy giving a job to an Italian, of all people. You may remember when the F story came out, Anderson wrote jokingly (I hope): how can you believe in bacterial sex, an incredible story told by an Irishman, an Italian and a Jew? I have forgotten which was the order in which he cited us, but it might say something about the order of unacceptability to British eyes of these ethnic groups.

I started by repeating your K-12 segregations. Fisher was very interested; his real desire was to have an organism for studying recombination and interference in large samples. Most if not all of his research was at the time on recombination and interference on mice and plants. He had immediately understood the effects of the segregation constraints on the recombination pattern in E.coli. When I added azide resistance, it was Norman Bailey who developed the max.likelihood estimation for it. None of us at the time understood the segregation pattern of sugars described by Newcombe (Amer. Naturalist 1949, I believe); it became clear to me only much later, and I developed their analysis in a 1954 paper with Jinks.

I decided to start studying the genetics of radioresistance and sensitivity, but it turned out that K-12 was not like B. I selected for nitrogen mustard resistance, and got Hfr (as well as an F- in 58-161, if I remember correctly; at least I presume it was). I immediately started working to make sure Hfr was really a frequency of recombination mutant and not something else. The person who was most sceptical when I spoke at a meeting in Cambridge in Spring '49 was Pontecorvo; he later invited me to repeat the K-12 experiments in Glasgow, in the presence of sceptical bacteriologists, which I did. Some bacteriologists of the time, especially from medical laboratories, said openly that bacterial geneticists were crazy. At Cambridge the biochemist E.F.Gale was cold; D.G.Catcheside looked at bacteria with suspicion, perhaps because he was used to the regular mendelian behavior of fungi. Fisher was the only person who was sympathetic and supportive.

My attempts at seeing the process of mating with Hfr were totally negative. I became more interested in outcrossing E.coli and, later, at studying quantitative inheritance to chloramphenicol. My position at Cambridge was not so secure; I was originally given a three years contract, and I felt pretty isolated scientifically, so when I was offered a good position back at the Sieroterapico

Milanesa, where a new director gave hopes of being able to develop the place into a reasonable research institution, I decided to return to Italy. I had spent two years in Cambridge, and left there Giulia Maccacaro whom I had called from Pavia and trained for a year, to continue the work.

This is the story of my beginnings in bacterial genetics. I was back in Milan in October 1950. Even with a new director from the U.S., the Sieroterapico remained, the mediocre place it always was. I had more freedom and more technicians but also more responsibilities. It was here, however, that I developed the story of F; I was trying to cross TLB1- to itself and developed new markers for this purpose. Untransformed TLB1- derived strains never crossed to each other, but all recombinants crossed freely. So I tried a menage a trois experiment and then direct infection, at the same time you did. The best visitor I had was John Jinks, with whom we found Hfr was linked to Gal; this was made public at the Genetics Congress of Bellagio in August 1953 but nobody noticed except Bertani. Somehow, Hayes managed to get much attention and publicity for the same observations on inheritance of Hfr done later; his streptomycin and UV experiments had got the limelights for him, and Paris circles advertised them widely.

I came to Madison in 1954, and we worked on sib selection in liquid medium. I also saw then your micromanipulation experiments with slim Hfr and fat F-cells, and was unable to see matings with the EM. My work back in Milan was not very productive. My interest started turning more and more heavily towards human genetics, after a slow beginning sometime during the early fifties. In early 1957 I left the Sieroterapico and started helping Adriano setting up Ph.D.-like courses in Pavia which were the prelude to the constitution of LIGB at Naples.

On my second visit to Madison, in 1958, we spent all the time trying to disprove the chromosome insertion theory of Wollmann and Jacob. We were not successful and yet it looked so improbable. Somehow, all the bacterial genetics work in Pavia between 1958 and 1960 was unsuccessful and the last work I did was with you and Esther, putting together joint observations on Gal suppression by streptomycin.

These reminiscences are probably much more than you asked; in retrospect, my worst mistake was to return to Italy. Had I spent one more year in Cambridge, I probably would have had the job promised by the University at the beginning, and would not have returned to a hopeless scientific desert. There was no way to stay competitive in the rapidly developing field of microbial genetics working in isolation in a place like the Sieroterapico, where I had limited mobility, increasing loss of scientific freedom and little or no chances of discussion. Had I stayed in Cambridge, a year or two later there would have been big local developments. Or I should have moved to the States. I left bacterial genetics too early to do anything substantive other than for the work done with you on F, and the Hfr work, which are often credited to Hayes only. The way things went, however, I did not do too badly in human genetics. The move to Stanford gave me a chance to go back to some experimental work, develop many new things, and be in the right place when the DNA revolution was ripe. I keep a letter from you in the early sixties in which you offered me a chair at Stanford. I accepted your invitation some ten years later. It is futile to speculate what I could have done if I had come to Stanford earlier. What I did in the sixties in Italy was limited to starting the work on Pygmies, and developing the Pavia lab and department which would otherwise have been destroyed. Adriano did not want to

leave anything in Pavia and tried hard to concentrate everything in Naples. I am glad I have saved Pavia from destruction. But I am somewhat sad of not seeing anything really first class come out of Pavia, or Naples, or indeed anywhere in Italian biology. There really are too few exceptions to this rule. No need to tell you this statement is highly confidential; I would not keep it confidential if it could change things, but I do not know what could be done about it.

I realise this letter is much too long and went beyond your expectations. But your question was provocative, and writing about it occupied pleasantly my time on the plane to, and back from Europe.

As ever,

Luca

Sept 30, 1986.